

DEPARTMENT OF PESTICIDE REGULATION
PEST MANAGEMENT GRANTS PROGRAM
Contract Number: 97-0221

Title: Augmentative Biological Control Using Transplants

Principal Investigator: Charles H. Pickett

Address: CDFA, 3288 Meadowview Rd., Sacramento, CA 95832

Telephone: (916) 262-2053 Fax: (916) 262-2059 e-mail: cpickett@cdfa.ca.gov

Principle Investigator: Greg Simmons

Address: USDA-APHIS PPQ, 4151 Hwy 86, Brawley, CA 92227

office phone: (760) 351-0532 Fax: (760) 344-7951

Disclaimer

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Department of Pesticide Regulation. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as actual or implied endorsement of such products.

Acknowledgements

The study reported herein was made possible through provision of cantaloupe acreage by privately owned farms in Imperial Valley. We are grateful to the Allen Bornt and Ralph Strahm family farms, Black Dog Farms, and Abbati Farms for allowing access to their land. This has been a cooperative project with laboratory facilities provided by the USDA-APHIS and Imperial Valley Whitefly Committee. The principal investigators are indebted to Enrique Lozano, the lead field technician, for his commitment and dedication to this project.

This report was submitted in fulfillment of contract # 97-0221, Augmentative Biological Control Using Transplants, by the California Department of Food & Agriculture under the partial sponsorship of the California Department of Pesticide Regulation. Work was completed as of May 26, 1999.

Table of Contents

| | Page |
|-----------------------------|------|
| Title Page..... | 1 |
| Acknowledgements..... | 2 |
| Table of Contents..... | 3 |
| Abstract..... | 4 |
| Executive Summary..... | 5 |
| Introduction..... | 6 |
| Materials and Methods..... | 8 |
| Results and Discussion..... | 10 |
| Conclusions..... | 11 |
| References..... | 12 |

Abstract

We are halfway through a three year field project demonstrating a novel technique for augmenting biological control of silverleaf whitefly. Recently imported *Eretmocerus* spp., parasitic Hymenoptera highly specific to whiteflies, have been successfully added to cantaloupe transplants. These plants were added to both organic and conventional fields of commercially grown cantaloupes in a desert growing region located in south-eastern California. Several obstacles to inoculating plants with adequate number of parasitized whiteflies have been overcome. In 1998, whitefly nymphs in a small plot (1/3 ac), replicated study in organic melons, were lowest in numbers in the transplant treated plots, followed by plots receiving parasites released by hand, and a no-release control plot. We have had difficulty in showing any impact in conventional fields due to low whitefly numbers. However, preliminary data in 1999 show that most parasites on transplants survived to adulthood in the imidacloprid treated plots.

Executive Summary

We have completed one field season and are part way into the second of a three field season project. Transplants of cantaloupe are being tested as a vehicle for augmenting a natural enemy of silverleaf whitefly, a serious pest of this field crop when grown in the desert region of southern California. Transplants of cantaloupe have been successfully inoculated with parasites and whiteflies and transferred into organic and conventional (use of imidacloprid for whitefly control) fields of cantaloupe. Several problems encountered in the inoculation of plants with parasitized whiteflies and in their placement in fields have been overcome. Host feeding of whiteflies by parasites and loss of young leaves on transplants resulted in lower than expected number of parasites added to fields. We added from 6,400 to 24,000 parasites per acre to fields using transplants, far less than the target of 40,000; the latter number has reduced whitefly numbers during previous hand release trials of parasites. Despite the low number of parasites added to the organic field in 1998, we observed differences in insect densities recorded in the three treatments: transplants, hand-release, and no-release control. On the last two sample dates, May 29th and June 9th, 1998, the transplant plots had the lowest numbers of nymphal whiteflies, compared to the other plots. The highest number of parasites recorded was in the hand-release plot on April 21, about one month after releases were made. The higher levels of parasitism and lower numbers of whitefly nymphs in the transplant plots suggests that this approach to releasing parasites is more effective than hand releases. Furthermore we ended up releasing about 9,000 parasites per acre more in the hand releases. In 1998 we were unable to compare the impact of parasites placed in imidacloprid treated plants due to the lack of whiteflies at this site. The combination of unusual weather, location in Imperial Valley, and effectiveness of imidacloprid prevented whiteflies from building to levels that could support parasites. Transplanting went well and our plants grew and matched the vigor of the grower's plants.

We are mid-year in our 1999 field season. Plants have been inoculated with parasites and placed in fields. We have set up a replicated, three treatment study at an organic field. We are comparing the impact of parasites released into ½ acre plots using either transplants or "hand releases" on silverleaf whitefly populations to a no-release control. The remaining three conventional fields use imidacloprid to control whiteflies and we set up paired transplant (inoculated with parasites), control plots in each. The cool spring is delaying growth of plants by 2 to 3 weeks. Cantaloupe will be harvested in late June and early July.

Introduction

The silverleaf whitefly (SLWF), *Bemisia argentifolii* (Homoptera: Aleyrodidae) Perring and Bellows, continues to be a serious pest of several vegetable and field crops in desert agricultural areas in California, as well as other regions of the United States. Major crops affected in California include melons, cotton, cole crops, alfalfa, and tomatoes (Perring et al. 1993). In 1993 and 1996, silverleaf whitefly reached damaging levels on cotton and in some instances citrus in the southern San Joaquin Valley, again raising fears that economically damaging populations of this pest are spreading to the more central regions of California's agricultural heartland. In the Imperial Valley alone, crop losses since 1991 are estimated at 330 million dollars (Birdsall et al. 1995). Additional losses in sales and employment in this region have been estimated at 630 million dollars. Damage nationwide, has been estimated at one billion dollars (Faust & Coppedge 1995).

The regional impact of silverleaf whitefly in Imperial Valley appears to have abated somewhat over the last two years due to the registration of imidacloprid (Admire™) in 1995 on some vegetable crops (section 18 for melons) and to changes in cropping patterns that disrupt continuity in whitefly host plants, i.e. the elimination of fall melon acreage. Most melon growers now use soil applications of imidacloprid that can provide 45-60 days of protection from whiteflies and other homopteran pests during spring months. However, given the silverleaf whitefly's capacity for rapid development of resistance to insecticides (Byrne et al. 1992, Prabhaker et al. 1992, Tan et al. 1996, Wolfenbarger & Riley 1994), and the demonstrated resistance to imidacloprid in laboratory studies (Prabhaker et al. 1995), other control strategies should be investigated.

Recent studies in Imperial and San Joaquin Valleys show that early season releases of parasites can suppress populations of silverleaf whitefly. Simmons et al. (1995, 1996) increased parasitism levels over twofold in silverleaf infested spring melons in commercial fields when making early season releases of the exotic parasites *Eretmocerus* spp. imported from India and Spain. Likewise, the density of whitefly nymphs was reduced by half. Similarly, Heinz et al. (1995) found a three fold reduction in silverleaf whitefly nymphs on cotton treated with releases of either *Eretmocerus* sp. imported from Texas or the native *Eretmocerus* from Imperial County. More importantly, augmentative releases may be used by growers treating cantaloupe with imidacloprid. In some fields, Simmons et al. 1996 released parasites following insecticide application, augmenting parasitism by two fold. Goolsby (unpubl. data) has demonstrated in replicated laboratory studies that late instar *Eretmocerus* larvae can survive field recommended rates of imidacloprid used on whitefly infested cantaloupe. Early season releases of parasites would increase the regional population of silverleaf whitefly parasites and possibly reduce or eliminate the use of pyrethroids used later in the season for additional control of adult whiteflies. Increases in the regional population of the highly specific whitefly natural enemy *Eretmocerus* spp. would benefit summer and fall crops susceptible to silverleaf whitefly.

Just as with many other efforts at using augmentative releases of natural enemies in field or vegetable crops (Oatman 1970, Pickett & Gilstrap 1986, Pickett et al. 1987), the

high cost of rearing the parasites needed for economic control of silverleaf whitefly may limit their use. In 0.5 acre plot studies, receiving ca. 40,000 parasites per acre (*Eretmocerus* ex. Pakistan), Simmons (unpubl. data) was able to maintain the same levels of parasitism in plots with and without applications of imidacloprid. He achieved similar levels of whitefly control when using imidacloprid and parasites, as imidacloprid plus the pyrethroid bifenthrin. These results suggest that field applied imidacloprid has little if no detrimental affect on released parasites and that the same releases can achieve similar levels of control as provided by bifenthrin. The parasites would cost growers \$200/ac who typically spend about \$120 per acre for whitefly control. We are proposing that the cost of augmentation can be reduced further, and efficiency increased, by using transplants inoculated with parasites. Rather than rearing whitefly and parasites on another non-crop plant, then harvesting and releasing the natural enemy, the crop itself is inoculated with parasites prior to its planting. Costs associated with growing non-crop plants are eliminated and mass rearing of parasites is reduced. Furthermore, fewer parasites introduced into a whitefly infested field on transplants may be needed to control whiteflies in a field receiving an equal number of hand released parasites. Hand released parasites must disperse over a large area, search for and then discover very low density prey while parasites emerging from inoculated plants would have prey readily available. The concept is simple, however no one has demonstrated the procedure, nor developed a protocol for inoculating plants and moving them into fields. Large numbers of transplants previously infested with small numbers of whiteflies can be inoculated with parasites in a greenhouse setting. These plants could be mixed with conventional transplants or seeded fields at planting. Although not widely practiced, there is a growing trend to using transplanted melons, versus those that are seeded. In addition to inoculating plants with a highly effective natural enemy, advantages to growers include reduction in costs due to seed loss, and reduction in water and pesticide usage.

We have almost completed the second of three field seasons in this study. The growing season for cantaloupe in Imperial Valley is February through June. We report on results completed in spring 1998 and on our 1999 season, which is still in progress. Both years we have conducted a study to compare the efficacy of inoculating fields with parasites using transplants vs. hand releases releases; and we have conducted a larger scale demonstration study in fields using imidacloprid.

Materials and Methods

Field Season 1998

Parasites were released into two commercial farms of cantaloupe in the Imperial Valley. The first was an organic grower, where we compared the effect of banker plants (transplants with parasites) against plots receiving hand-releases of parasites, and a no-release control. Treatments were assigned to 1/3 ac plots using a randomized complete block design with 4 replicates. The second site was a conventional grower who uses imidacloprid (Admire®), and we compared whitefly plant densities in 2 pairs of 1 acre plots with and without the addition of banker plants, respectively. Approximately 20,000 adult wasps were used to inoculate a flat of 196 transplants, each bearing approximately 100 whitefly eggs on their first and second true leaves.

Field Season 1999

A three treatment, replicated study was set up at a 32-ac organic farm site. We are repeating a study in which the efficiency of release methods is being compared in an insecticide free environment. Plots of cantaloupe receiving parasite-inoculated transplants are being compared to plots with either "hand released" parasites or a no-release control. We were able to increase the size of our study sites over last year from 1/3 ac to 1/2 ac. In addition, plots were separated by at least one acre, whereas last year we had no separation. We succeeded in adding 24 parasite pupae per transplant, or 12,000 per ac, up from ca. 9 per plant last year (Table 1). Our goal was 40,000 parasites per acre, however since the regional whitefly population is again very low, these numbers are most likely adequate for economical control. Last year far fewer parasite pupae were added to transplants due to host feeding by adult parasites used in the inoculation process. A 10:1 whitefly to parasite ratio was used this time, up from a 1:1 ratio used last year. We released on April 28 the same number of adult wasps in the "hand release" plots using small paper cups, five weeks after "banker" plants were added to the transplant plots. The first generation silverleaf whitefly nymph population was just getting established at this time. The organic producer used a dripline under black plastic, and his transplants went in on March 15th, one week before ours. Our transplants are identical in size and vigor as the grower's.

We had difficulties getting our conventional fields started, but managed to get inoculated plants into 3 fields using imidacloprid, and two of these were on drip line. One of our original growers delayed planting and a second backed out at the last minute. As a result, we were late in getting our cantaloupe transplants into the fields. We set up paired, 1-acre treatment and control plots at each of these sites. All three of these growers planted by seed, and two of the three used dripline under plastic until plants were well developed, after which plastic was removed. Imidacloprid is added through the dripline. Our transplants had to be replanted in two of these fields because they died due to water stress and heavy winds. Fewer parasites were added to transplants used in these fields, 4 – 6.55

per plant, or about 2,500 parasites per acre. This again is much lower than we had planned initially, but the whitefly numbers were so low in these fields that these should provide adequate control.

Results and Discussion

Field Season 1998

We succeeded in getting parasites onto banker plants and into fields at both the organic and conventional fields. About 10% of the melon plants in plots receiving banker plants were inoculated with parasites. However, we ended up releasing far fewer parasites using banker plants than we had planned; about 6400 to 7800 parasites per acre at the organic farm and approximately 24,000 per acre at the conventional field. This is much lower than our target of 40,000, the number found to give good control of whiteflies using conventional hand releases. Nevertheless, we measured significant differences in whitefly nymphal populations between the different treatment plots at the organic site. The lowest nymphal populations on the last two sample dates were recorded from the transplant plots, with increasing number in the hand release, and control plots (Fig. 1). On 29 May 1998 banker plant plots averaged 0.13 nymphs/cm² followed by hand release plots at 0.18 nymphs/cm², and control plots, 0.23 nymphs/cm²; and on 9 June 1998 bankers plant plots averaged 0.28 nymphs/cm² followed by hand release plots at 0.41 nymphs/cm², and control plots 0.51 nymphs/cm². We detected few whiteflies at the conventional field receiving an imidacloprid treatment. Parasitism remained extremely low the entire season in both treatments, most likely as a result of a rare host population.

Field Season 1999

We are mid-way in our 1999 field season. We reared parasites and inoculated transplants for four commercial fields of cantaloupe during early spring 1999. Transplants were successfully planted at one organic field and into 3 conventional fields. We added enough plants to fields so that 10% of all would be inoculated with parasites.

Only about 5 to 10% of the eggs on transplants resulted in parasitized whiteflies. Most of this attrition was due to egg mortality. Studies just done last year show that 30 to 50% of eggs die before molting to first instars (Naranjo, pers. Comm. 1998). Our highest number of parasitized whiteflies occurred on transplants with the highest number of whitefly eggs suggesting we must increase our transplant egg load to increase the final number of parasites on these plants going into the field. The similarity in the number of parasitized whitefly on both the transplants sampled from the field, and those held back at the field station at time of planting (Table 1), suggest that the imidacloprid had very little to no impact on the insects. Plants are now growing well at all three conventional fields, but smaller in size compared to the grower's. Whitefly numbers are extremely low at these three sites.

Conclusions

A protocol for inoculating melon transplants with parasites highly specific for silverleaf whitefly has been developed. Our first year of a replicated field study showed that these plants can raise the population level of parasites and whitefly parasitism rates. Fields receiving parasites through this method had lower whitefly numbers than fields receiving parasites by hand, i.e. pupae filled cups placed in plots, and no-release control plots. These results suggest that augmentation, using transplants, is more efficient than releasing by hand. We have also found high larval parasite survivorship on transplants placed in fields receiving imidacloprid through drip lines

References:

- Birdsall, S. L. D. Ritter, & P. L. Cason. 1995. Economic impact of the silverleaf whitefly in the Imperial Valley, California, p. 162. *In* T. J. Henneberry, N. C. Toscan, R. M. Faust, and J. R. Coppedge (eds.), Silverleaf whitefly (formerly sweetpotato whitefly, strain B): 1995 supplement to the 5-year national research and action plant third annual review held in San Diego, California, January 28-31, 1995. U.S. Department of Agriculture, 1995: 305 pp.
- Byrne, F. J., I. Denholm, L. C. Birnie, A. L. Devonshire, and M. W. Rowland. 1992. Analysis of insecticide resistance in the Whitefly, *Bemisia tabaci*, pp. 165-178. *In* Denholm, I. A. L. Devonshire and D. W. Hollomon [eds], Resistance' 91: Achievements and developments in combating pesticide resistance. Elsevier, London.
- Faust, R. M. & J. R. Coppedge. 1995. Foreword, p. iii *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge (eds.), Silverleaf whitefly (formerly sweetpotato whitefly, strain B): 1995 Supplement to the 5-year National Research and Action Plan, San Diego, California, January 28-31, 1995. U.S. Department of Agriculture-ARS, 1995: 305 pp.
- Freund, R. J. R. C. Littell, and P. C. Spector. 1986. SAS System for Linear Models. SAS Institute Inc., Box 8000, SAS Circle, Cary, NC.
- Heinz, K. M., J. R. Brazzle, J. A. Brown, K. A. Casanave, and C. H. Pickett. 1995. Evaluations of *Eretmocerus* nr. *californicus* on cotton in the San Joaquin Valley, *In* L. G. Bezark (ed.) Biological Control Program Annual Summary, 1994. California Department of Food and Agriculture, Division of Plant Industry, Sacramento, California. 51 pp.
- Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, and T. S. Bellows, Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. *Science* 259: 74-77.
- Oatman, E. R. 1970. Integration of *Phytoseiulus persimilis* with native predators for control of the two-spotted spider mite on rhubarb. *J. Econ. Entomol.* 63: 1177-1180.
- Pickett, C. H., and F. E. Gilstrap. 1986. Inoculative releases of Phytoseiidae (Acari) for the biological control of spider mites (Acari: Tetranychidae) infesting corn. *Environ. Entomol.* 15: 790-794.
- Pickett, C. H., F. E. Gilstrap, R. K. Morrison, and L. F. Bouse. 1987. Release of predatory mites (Acari: Phytoseiidae) by aircraft for the biological control of spider mites (Acari: Tetranychidae) infesting corn. *J. Econ. Entomol.* 80: 906-910.
- Prabhaker, N., N. C. Toscano, T. M. Perring, G. Nuessly, K. Kido, and R. R. Youngman. 1992. Resistance monitoring of the sweetpotato whitefly (Homoptera: Aleyrodidae) in the Imperial Valley of California. *J. Econ. Entomol.* 85: 1063-1068.

- Prabhaker, N. N. C. Toscano, S. Castle, & T. Henneberry. 1995. Hydroponic bioassay technique to monitor responses of whiteflies to imidacloprid, p. 89. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge (eds.), Silverleaf whitefly (formerly sweetpotato whitefly, strain B): 1995 Supplement to the 5-year National Research and Action Plan, San Diego, California, January 28-31, 1995. U.S. Department of Agriculture-ARS, 1995: 305 pp.
- Simmons, G. S., K. Hoelmer, R. Staten, and T. Boratynski. 1995. Biological control of *Bemisia* in spring melons. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge (eds.), 1995 Supplement to the 5-year National Research and Action Plan, San Diego, CA January 28-31, 1995. U.S. Department of Agriculture, Agricultural Research Service, 1995-02, 299 pp.
- Simmons, G. S., K. Hoelmer, R. Staten, and T. Boratynski. 1996. Seasonal inoculations in spring melons with parasitoids of *Bemisia*. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge (eds.), 1996 Supplement to the 5-year National Research and Action Plan, San Antonio, Texas, February 4-6, 1996. U.S. Department of Agriculture, Agricultural Research Service, 1996-01, 232 pp.
- Tan, W., D. G. Riley, and D. A. Wolfenbarger. 1996. Quantification and genetic analysis of bifenthrin resistance in the silverleaf whitefly. *Southwest. Entomol.* 21: 265-275.
- Tonhasca, A. Jr., J. C. Palumbo, and D. N. Byrne. 1994. Distribution patterns of *Bemisia tabaci* (Homoptera: Aleyrodidae) in cantaloupe fields in Arizona. *Environ. Entomol.* 23: 949-954.
- Wolfenbarger, D. A., and D. G. Riley. 1994. Toxicity of mixtures of insecticides and insecticides alone against b-strain sweetpotato whitefly, pp. 1214-1216. *In* D. J. Herber and D. A. Richter [eds.], Proceedings, Beltwide Cotton Production Conference, San Antonio, TX.

Fig. 1. Organic melons, Imperial Valley, 1998.
Effect of different release methods. Means \pm 1 SE.

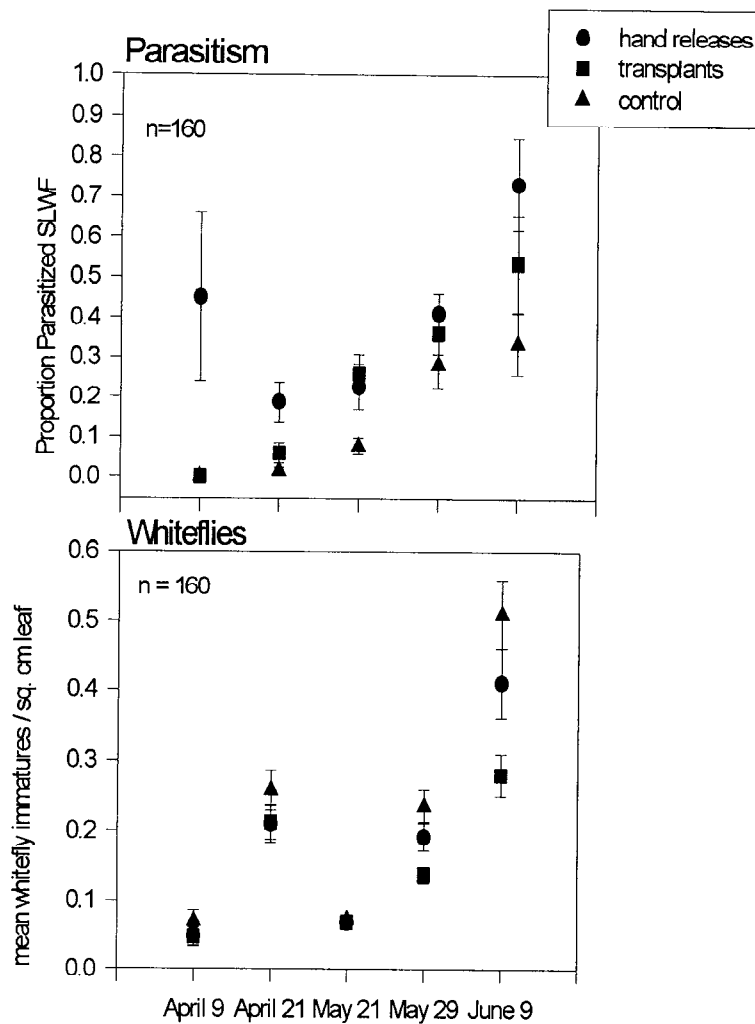


Table 1. Numbers of insects on transplants placed in cantaloupe fields, spring 1999.

| Field | Mean insects/transplant | | | Per cent of whiteflies parasitized on transplants in field | Mean #eggs/transplant at inoculation |
|-----------|--|--|---|--|--------------------------------------|
| | Parasites on plants held back ¹ | Parasites on transplants put in field ¹ | Whiteflies on transplants put in field ² | | |
| Bornt | -- | 24.38 | -- | 84 | 259 |
| Strahm | 2.68 | 4.03 | 8.51 | 88 | 142 |
| Black Dog | -- | 6.55 | 4.6 | 90 | 160 |
| Abatti | 7.1 | 7.6 | 4.24 | 80 | 149 |
| | | | | | |

¹parasite pupae plus exit holes, five weeks after placement in fields²live and dead fourth instars, plus whitefly exit holes, five weeks after placement in fields